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Evaluation of the Safety and Pharmacokinetic Profile of a New, Pasteurized, Human Tetanus Immunoglobulin Administered as Sham, Postexposure Prophylaxis of Tetanus

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In a monocentric, double-blind, randomized trial, we examined the safety and pharmacokinetic profile of a new, pasteurized, human tetanus immunoglobulin (P-HTIG). As part of the purification process, P-HTIG has undergone a heat treatment step (10 h at 60°C) and the removal of Merthiolate. Forty-eight adults with a history of tetanus vaccination were randomized into four groups ($n = 12$ per group) to receive one of two different batches of this P-HTIG simultaneously with either tetanus-diphtheria (Td) vaccine (sham, postexposure prophylaxis of tetanus) or placebo. Local reactions at the injection site were followed for the first 3 days after injection, and systemic reactions were followed during the entire study period, i.e., up to 42 days posttreatment. Blood samples for tetanus antibody titer determination (enzyme-linked immunosorbent assay method) were drawn prior to treatment on day 0 and on days 1, 2, 3, 7, 14, 21, 28, 35, and 42. A normalization of tetanus antibody titers (subtraction of the day 0 value for each subject at each time period) was performed to assess the additive effect of P-HTIG on tetanus antibody titers. The pharmacokinetic parameters were determined by both a compartmental analysis (modelization) and a noncompartmental analysis. No severe adverse reactions were reported. The rate of local reactions at the P-HTIG injection site was 27%. All local reactions were mild and resolved within 2 days. In contrast, local reactions at the vaccine injection site were seen in 79% of the subjects. The rate of systemic reactions was similar in the P-HTIG plus Td vaccine group (33%) and in the P-HTIG plus placebo group (21%), and all these reactions were mild. In the P-HTIG plus placebo group, tetanus antibody titers rose to a maximum of 0.313 ± 2.49 IU/ml after 4.4 days; in the P-HTIG plus Td vaccine group, a maximum concentration of 15.2 ± 2.42 IU/ml was reached 19 days postinjection. In both groups, 100% of the patients had seroprotective levels of tetanus antibodies (≥0.01 IU/ml) 2 days following treatment. An anamnestic response to Td vaccine appeared 7 days postimmunization. In conclusion, P-HTIG has a good safety and pharmacokinetic profile. Our results confirm that immunoglobulin should be associated with vaccine in the treatment of tetanus-prone wounds.

Tetanus is a serious neurological disease characterized by severe muscular spasms. It is caused by the neurotoxin produced by the anaerobic bacterium *Clostridium tetani* in a contaminated wound. Due to high rates of vaccine coverage, tetanus morbidity and mortality have decreased dramatically in developed countries. However, tetanus remains a major public health problem in most developing countries. The worldwide annual incidence of fatal cases of tetanus has been estimated to be close to 1 million individuals, with 80% of the fatal cases of tetanus occurring in neonates (3, 26). In 1995 at least 450,000 deaths were due to neonatal tetanus (38). Nonneonatal tetanus occurred in 500,000 people, with a death rate of about 40% (26). In spite of medical control of convulsions and assisted ventilation, the fatality rate is still elevated (range, 7 to 58%) even in countries with high medical standards and especially in older patients (21, 24). Prevention remains the best means of reducing the incidence of and mortality from tetanus.

Treatment of wounds at risk for tetanus infection consists of active immunization (vaccine), local wound management, and passive immunization (immunoglobulin). Passive immunization, which was initially developed at the beginning of the 20th century, is still relevant today for prophylactic treatment of patients with tetanus-prone injuries whose immunity is either incomplete or unknown. It is also used in the treatment of patients with tetanus. The current recommendations for tetanus wound prophylaxis are indicated in Table 1 (4).

Specific serum was originally prepared from immunized animals, but severe adverse reactions occurred, such as early anaphylactic or late serum sickness reactions (35) related to the use of unrefined, heterologous, crude serum. In addition, a protective level was maintained for only 1 to 2 weeks (34), and doses of 1,500 to 3,000 IU were required. All these issues led to the subsequent development of human tetanus immunoglobulins, which at its current recommended dose of 250 to 500 IU has proven to be well tolerated and provides better protective levels of circulating antibodies for much longer periods than does heterologous immunoglobulin (34).

The human tetanus immunoglobulin (Tetaglobuline) from Pasteur Mérieux Connaught has been manufactured for more than 20 years from human plasma with high tetanus antibody titers by a fractionation and purification method based on the process of Cohn, a method with a proven capacity to eliminate and inactivate viruses (37). To further improve safety by reducing the probability of virus passage via immune globulin administration, a pasteurization step has been added to the manufacturing process. The bulk solution of the purified tetanus immunoglobulin is heated for 10 h at 58 to 60°C. In addition, Merthiolate (a preservative) was removed.

The purpose of the present study was, first, to evaluate the

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TABLE 1. Recommendations for tetanus wound prophylaxis[a]

Type of wound	Patient not immunized or partially immunized	Patient completely immunized, with the last booster dose received the following no. of years earlier:	
		5–10 yr	>10 yr
Clean, minor	Begin or complete immunization per schedule; tetanus toxoid, 0.5 ml	None	Tetanus toxoid, 0.5 ml
Clean, major or tetanus prone	In one arm, HTIG at 250 U; in the other arm, tetanus toxoid at 0.5 ml and complete immunization per schedule	Tetanus toxoid, 0.5 ml	In one arm, HTIG at 250 U; in the other arm, tetanus toxoid at 0.5 ml
Tetanus prone; delayed or incomplete debridement	In one arm, HTIG at 500 U; in the other arm, tetanus toxoid at 0.5 ml and complete immunization per schedule; antibiotics	Tetanus toxoid at 0.5 ml; and in the other arm antibiotics	In one arm, HTIG at 500 U; in the other arm, tetanus toxoid at 0.5 ml and antibiotics

[a] Data are from a previous report (4).

local and systemic safety profile of this product, pasteurized human tetanus immunoglobulin (P-HTIG), in healthy volunteers. It was administered alone or as part of a sham postexposure prophylaxis for tetanus (i.e., concurrent administration of P-HTIG and tetanus-diphtheria [Td] vaccine to patients who had not received a tetanus booster in the previous 10 years). The posttreatment tetanus antibody titers were characterized, and the pharmacokinetic parameters of P-HTIG, alone or in association with the tetanus vaccine, were determined. The combined Td vaccine was used because subjects immunized because they have a tetanus-prone wound are likely to benefit from a diphtheria booster. Indeed, a low seroprevalence of diphtheria antibodies has been reported in adults (19).

MATERIALS AND METHODS

Study population. During 1995, 48 subjects were selected at INNOVEX Biodesign, Freiburg, Germany. All subjects gave written consent after having been informed of the nature of the trial and its potential risks. This trial was conducted in accordance with the latest revision of the Declaration of Helsinki, with European good clinical practices, and with local regulatory requirements. The protocol was approved by an institutional review board (INNOVEX).

(i) Inclusion criteria. Adults ages 18 to 50 years who were of either sex and in good health and who had no clinically significant abnormalities by medical or clinical laboratory examination were included in the study. Each subject had a documented history of primary immunization against tetanus (three injections), with receipt of the last tetanus booster dose dating back at least 10 years.

(ii) Exclusion criteria. A past history of renal or hepatic disease or of an uncontrolled coagulopathy, a positive human immunodeficiency virus, hepatitis B virus surface antigen, or hepatitis C virus serology, a positive urine test for pregnancy, a febrile illness (oral temperature, ≥37.5°C) at the time of vaccination, a history of a severe adverse reaction or allergy to any component, or treatment with immunosuppressive therapy (including corticosteroids) within the past month or a treatment that increases hepatic microsomal enzyme activity prevented a subject from participating in the study.

Study design. The present study was a monocentric, double-blind, randomized trial. During a screening visit (within the 14 days preceding treatment), the investigators selected subjects who fulfilled all of the inclusion criteria and who had none of the exclusion criteria. The health status of each subject was determined by medical history and by a physical examination (including electrocardiogram, blood pressure, and pulse rate) as well as laboratory testing for hepatitis B virus surface antigen, human immunodeficiency virus types 1 and 2, and hepatitis C virus antibodies, a complete blood count and serum chemistry, a urinalysis, and a pregnancy test (for women).

On day 0, after again checking the inclusion and exclusion criteria, the subjects were randomized into the following groups: group A (n = 12), P-HTIG (500 IU; batch S3113) and placebo vaccine; group B (n = 12), P-HTIG (500 IU; batch S3115) and placebo vaccine; group C (n = 12) P-HTIG (500 IU; batch S3113) and Td vaccine; and group D (n = 12), P-HTIG (500 IU; batch S3115) and Td vaccine. A pretreatment blood sample for tetanus antibody titration was obtained. All subjects received P-HTIG (500 IU) and one dose of either Td vaccine or placebo. They were followed for 30 min to detect any immediate reactions. The subjects were then given a self-monitoring form to record local or systemic symptoms.

Subjects returned for follow-up visits on days 1, 2, 3, 7, 14, 21, 28, 35, and 42, during which safety was evaluated and blood samples were obtained. Hematological and biochemical determinations, as well as urinalysis (as at the screening visit), also were performed on day 42.

P-HTIG and Td vaccine administration. (i) Products. P-HTIG, vaccine, and placebo were manufactured by PMC, Lyon, France. The composition of P-HTIG was as follows: tetanus immunoglobulin, not less than 250 IU/ml; glycine, 20 mg/ml; sodium chloride, 1 mg/ml; and water for injection (up to 1 ml). The product was presented in a glass ampoule of 1.0 ml. Two batches were compared in this study: S3113 (batch 1) and S3115 (batch 2).

The composition of the Td adsorbed vaccine was as follows: purified diphtheria toxoid, not less than 2 IU per single human dose; purified tetanus toxoid, not less than 20 IU per single dose; aluminum hydroxide, a maximum of 1.25 mg; sodium mercurothiolate (preservative), a maximum of 0.05 mg; and 0.9% sodium chloride solution, up to 0.5 ml. The product was presented in a glass syringe of 0.5 ml.

The composition of placebo was as follows: sodium chloride, 4.15 mg; disodium hydrogenate, 0.065 mg; sodium dihydrogenate, 0.023 mg; and water for injection, up to 0.5 ml. The product was presented in a glass syringe of 0.5 ml.

(ii) Route of administration. P-HTIG (2 ml; 500 IU [the content of two 1-ml ampoules of 250 IU each]) was injected slowly into the left deltoid by the intramuscular route. A single, 0.5-ml dose of Td vaccine or placebo was injected into the right deltoid muscle by the intramuscular route. Although the packaging was identical for the adsorbed Td vaccine and the placebo vaccine, there is a difference in appearance of vaccine and placebo. Thus, to ensure blinding, the products were administered by a nurse who was not involved in the safety assessment of subjects.

Determination of tetanus antibody levels. Blood samples (5 ml) for determination of tetanus antitoxin levels, taken on day 0 (D0) (just before injection of immunoglobulin and vaccine) and on D1, D2, D3, D7, D14, D21, D28, D35, and D42, were collected in dry tubes under sterile conditions. Each sample was centrifuged (2,000 × g for 15 min at 4°C), and the serum was divided into two aliquots, correctly labelled, and frozen at −20°C. Antitetanus antibodies in human serum were assayed by an enzyme-linked immunosorbent assay (ELISA) procedure (18, 31). A monoclonal, peroxidase-labelled antibody specific for human antitetanus immunoglobulin G was used. Results were expressed in international units per milliliter. The sensitivity was 0.006 IU/ml, and the range of the assay was 0.0015 to 0.05 mIU/ml. Serum was diluted for samples with antibody concentrations of greater than 0.05 mIU/ml. The coefficient of interassay reproducibility was 20%.

Subjects with a tetanus antibody titer of <0.01 IU/ml were considered to be not protected; those with a tetanus antibody titer in the range of 0.01 and up to (but not including) 0.1 IU/ml were considered to have possible, but uncertain, seroprotection; those with a titer of ≥0.1 IU/ml were considered to be seroprotected (9).

Safety analysis. All immediate reactions occurring within 30 min of injection, local reactions occurring from 30 min to 72 h after injection, and systemic reactions occurring from D0 to D42 were recorded. During the study, safety information was collected by the subjects on the self-monitoring form and then by the investigator at each visit. Subjects were questioned about any adverse event, and a physical examination was performed. The local and systemic adverse reactions were classified by severity, date of onset, and duration. Adverse events were classified according to their severity as mild (i.e., the subject was aware of the symptoms but they were easily tolerated) or moderate or severe (i.e., they were discomforting enough to interfere with normal daily activity or to cause disability). Fever (oral temperature, ≥37.5°C but <38.5°C or ≥38.5°C), pain (tenderness to touch or pain upon movement), erythema, swelling or induration

TABLE 2. Characteristics of the four study groups at time of inclusion in the study

Group	Age (yr)[a]	Ht (cm)[a]	Wt (kg)[a]	Male/female ratio	Time since last tetanus booster (yr)[a]
A	34.0 ± 10.2 (20–47)	170 ± 9.0 (157–185)	70.0 ± 9.1 (58–83)	1:1.4	16.9 ± 5.5 (10–26)
B	31.0 ± 7.3 (21–48)	174 ± 6.6 (162–183)	66.0 ± 8.7 (54–81)	1:2	17.6 ± 6.5 (10–30)
C	36.0 ± 4.1 (31–42)	172 ± 8.9 (158–187)	72.0 ± 13.4 (54–94)	1:1	15.1 ± 3.4 (11–21)
D	30.7 ± 5.3 (21–42)	171 ± 7.7 (162–172)	71.1 ± 10.3 (59–94)	1:2	17.2 ± 6.0 (10–28)

[a] Data are expressed as means ± SDs (minimum-maximum values) (n = 12 subjects in each group).

(2.5 cm or greater), and regional adenopathy (1 cm or greater) were recorded in a separate category. Erythema and swelling or induration were measured with a caliper.

Also recorded were the number and percentage of subjects in each group with one or more local reactions at the injection site, i.e., pain, erythema, pruritus, swelling or induration, or regional lymphadenopathy, and the number and percentage of subjects presenting with one or more systemic reactions, i.e., urticaria, rash, malaise, arthralgia, headache, or gastrointestinal disorders such as nausea, vomiting, diarrhea, or abdominal pain, following treatment.

Pharmacokinetic analysis. Geometric mean titers (GMTs) were calculated by day of postinjection sampling from individual measurements of levels of tetanus antibodies in serum for each subject group. The GMT of antibody titers T_1, T_2, T_3, . . . T_N was calculated according to the following equation: GMT = antilog (Σ log T_i/N), where T_i is the antibody titer for subject i, and N is the number of subjects with antibody titer. Descriptive data on the levels of tetanus antibodies in serum were given for the four groups, and evolution curves were obtained with the 10 datum points obtained on D0 to D42.

Since baseline antibody titers were elevated, the effects of P-HTIG (alone or with Td vaccine) on tetanus antibody titers were not evident from the raw primary data. Hence, to determine the increase in antibody titers from the baseline due to the products for each subject, a procedure consisting of the subtraction of the baseline titers from all postimmunization antibody titers was carried out.

The following pharmacokinetic parameters were then determined for each study group by a noncompartmental analysis (1, 2, 5, 13). (i) For the area under the serum concentration-time curve (AUC), two parameters were used for the area calculation. AUD is the area under the curve for the concentration-versus-time profile up to the last quantifiable datum point, i.e., D42. It was calculated by the linear trapezoidal rule. AUC is the area under the curve extrapolated to infinity by using the calculated datum point at the last time point of the last quantifiable concentration according to the equation AUC = AUD + C_t/k_{el} (where C_t is the last quantifiable concentration in serum and k_{el} is the elimination rate constant). (ii) The maximum concentration in serum (C_{max}) was obtained directly from the concentration-time profile. (iii) The time to C_{max} (T_{max}) was also obtained directly from the concentration-time profile. (iv) The time to reach titers of 0.01 IU/ml (minimum seroprotective level) and 0.1 IU/ml were also determined. Apparent clearance and volume of distribution could not be calculated because of the limited number of datum points. Although the normalization and selection processes do not reflect the real evolution of antibody titers in an individual subject (especially for those subjects with high baseline antibody levels), a model for the decline in titers to the baseline level with time can be postulated. Hence, a compartmental analysis with modelization was used to describe the antibody response after administration of P-HTIG alone or simultaneously with Td vaccine. In addition, this model would be useful for forecasting increases in antibody titers after administration of P-HTIG alone or with vaccine. This model is described in the appendix.

Statistical analysis. All values are expressed as means ± standard deviations (SDs) (or standard errors [SEs] for Fig. 1 and 2). Descriptive analysis was used for the safety results. The pharmacokinetic calculations were carried out by using TopFit (12). The weight scheme for the modeling procedure was $1/y^2$ for the P-HTIG (S3113 or S3115) plus Td vaccine group and 1 for the P-HTIG (S3113 or S3115) plus placebo group. The weighting with $1/y^2$ for the first set of data was chosen to force the fitting algorithm to consider the small concentrations during the first 3 days adequately. An unpaired Student's t test was used only to determine differences in pharmacokinetic parameters between the two P-HTIG batches. All statistical evaluations were performed with SAS software (30).

RESULTS

A total of 48 subjects (12 per group) were included in the study, and all subjects completed the study. Age, height, weight, male/female ratio, and time since last tetanus booster (summarized in Table 2) did not differ between groups.

No serious adverse events, in particular, no anaphylactic reactions, were reported. A subject in group D developed dizziness and nausea, with symptoms that were of short duration (8 min) and of mild intensity. Table 3 presents the number and percentage of subjects with reactions (local and systemic) after administration of P-HTIG alone or in association with Td vaccine. Local reaction rates at P-HTIG injection sites were similar (21% in groups A and C versus 33% in groups B and D), demonstrating the absence of a batch effect. Pain was the most frequently reported local reaction. Overall, all local reactions to P-HTIG except pruritis occurred within the first day after the injection; pruritis occurred 34 h after injection in a subject in group D. All reactions were mild in intensity and, with one exception (pain persisting for 2.5 days in a subject in group D), lasted no more than 2 days.

The percentage of subjects with local reactions was markedly greater in groups given Td vaccine (79% in groups C and D versus 4% in groups A and B). Again, pain was the most frequently reported reaction. All these reactions were mild except in one subject (pain, moderate; group C). Thirteen reactions (13 of 21; 62%) lasted more than 48 h.

Since there was no difference between the two batches, the systemic reactions of subjects in groups A and B and those of subjects in groups C and D were pooled and are summarized in Table 3. Systemic reaction rates were similar in the P-HTIG plus Td vaccine group (33%) and in the P-HTIG alone group (21%). These reactions were mild in all except one subject (herpes labialis, moderate; group D). Most of the reactions were reported within the 2 days following injection; all reactions except herpes labialis and ganglion swelling disappeared within 2 days. Finally, pre- and postimmunization physical examinations (i.e., vital signs and electrocardiogram on D42) and clinical examinations revealed no clinically significant abnormalities in either group.

The evolution of tetanus antibody titers up to 42 days postinjection are presented in Fig. 1 (groups A and B and groups C and D). Baseline (D0) antibody titers were elevated in the four groups (range, 0.05 to 10.03 IU/ml). However, 12% of the subjects had antibody titers below 0.1 IU/ml and were possibly unprotected. In the groups receiving P-HTIG plus placebo (groups A and B), the GMT rose from 1.40 ± 3.58 IU/ml (D0) to a maximum value of 1.63 ± 2.96 IU/ml (D2) in group A and from 1.31 ± 7.27 IU/ml (D0) to a maximum value of 1.62 ± 3.90 IU/ml (D3) in group B.

Then antibody titers decreased gradually, and by day 28 they were 1.45 ± 3.47 IU/ml in group A and 1.49 ± 4.64 IU/ml in group B. For those given P-HTIG plus Td vaccine (groups C and D), the GMT rose from 2.23 ± 3.36 IU/ml (D0) to a maximum value of 10.78 ± 1.99 IU/ml (D21) in group C and from 0.63 ± 4 IU/ml to 16.49 ± 2.31 IU/ml (D14) in group D. For groups C and D, the slope of the titer curve changed dramatically between D3 and D7, probably because of antibody production in response to the vaccine. The GMT at day 28 was 9.07 ± 1.80 IU/ml in group C and 14.92 ± 1.76 IU/ml in group D.

Although the patients had received the last tetanus vaccination more than 10 years earlier, baseline tetanus antibody titers

TABLE 3. Numbers of subjects with one or more reactions after administration of P-HTIG alone or in association with Td vaccine

Type of reaction, site, and reaction	No. (%) of subjects				Mean ± SD delay of onset (h)		Duration (h)	
	P-HTIG batch 1 (groups A and C; $n = 24$)	P-HTIG batch 2 (groups B and D; $n = 24$)	P-HTIG plus placebo (groups A and B; $n = 24$)	P-HTIG plus Td vaccine (groups A and B; $n = 24$)	P-HTIG (all batches) plus placebo	P-HTIG plus Td vaccine	P-HTIG plus placebo	P-HTIG plus Td vaccine
Local reaction								
P-HTIG site								
Pain	4 (17)	7 (29)			6 ± 6.5		19 ± 16	
Induration or swelling	2 (8)	0			4.5 ± 7		14 ± 4	
Pruritis	1 (4)	1 (4)			18 ± 22		9 ± 4	
Any local reaction	5 (21)	8 (33)						
Td vaccine or placebo site								
Pain			1 (4)	19 (79)		18 ± 13		100 ± 66
Pruritis			0	2 (8)		27 ± 28		129 ± 181
Redness			0	1 (4)		26		150
Any local reaction			1 (4)	19 (79)				
Systemic reaction								
Dizziness				1 (4)		0		0.2
Headache			4 (17)	4 (17)	35 ± 58	55 ± 69	3.6 ± 2.0	18 ± 13
Fever				1 (4)		133		33
Fatigue			1 (4)		23		13	
GITD[a]				3 (12)		15 ± 18		20 ± 18
Other reactions[b]				5 (20)		421 ± 339		226 ± 187
Any systemic reaction			5 (21)	8 (33)				

[a] GITD, gastrointestinal tract disorder (see text).
[b] Other reactions were chills, arthralgia, herpes labialis, and ganglion swelling.

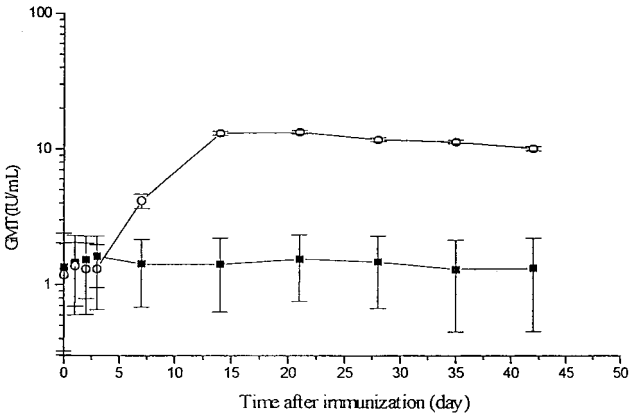

FIG. 1. Evolution of tetanus antibody titers (mean ± SE; log-linear scale) after administration of P-HTIG plus placebo (groups A and B) (■, $n = 24$) or P-HTIG plus Td vaccine (groups C and D) (○; $n = 24$).

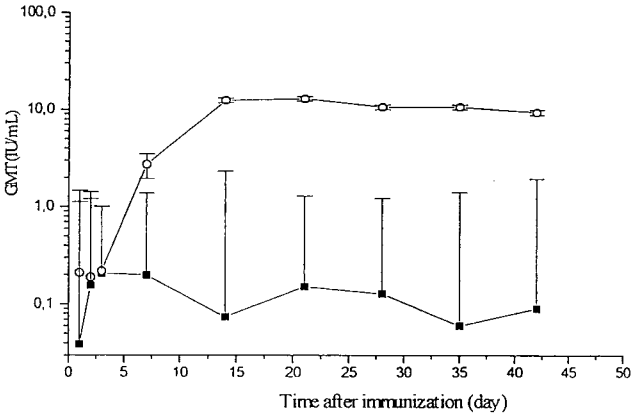

FIG. 2. Evolution of tetanus antibody titers (mean ± SE; log-linear scale; data normalized) after administration of P-HTIG plus placebo (groups A and B) (■; $n = 6$) or P-HTIG plus Td vaccine (groups C and D) (○; $n = 13$).

were high and it was difficult to describe the effect of P-HTIG. Hence, a procedure was used to determine the individual increase in antibodies titers from the baseline due to the administered products. After this correction, it was expected that the antibody titers were positive during the entire follow-up period. However, the baseline titer was not the lowest value measured for some subjects, probably because of the masking effect of assay variability at high baseline concentrations on the passive titers induced by P-HTIG (from 0.05 to 0.2 IU/ml at this dose) (8). Since only antibody titer profiles with positive titers showed the real effects of the administered products, only these were subjected to a separate, noncompartmental analysis. The number of evaluable subjects (or profiles) was less than the total number of subjects (placebo groups A and B, $n = 6$; Td vaccine groups C and D, $n = 13$). Figure 2 shows the evolution of the tetanus antibody titers during the 42-day period following immunization for groups A and B and groups C and D. The two curves have similar profiles until D3 to D7. There was a high intersubject variability in the response to P-HTIG injection.

Since no difference in results was found between the two batches, pharmacokinetic parameters were determined for groups A and B (P-HTIG plus placebo) combined and for groups C and D (P-HTIG plus Td vaccine) combined.

The pharmacokinetic results are summarized in Table 4. On account of the variability of titers, it was difficult to determine k_{el}, and only AUD was determined for most subjects in all of the groups. In addition, for those receiving immunization with vaccine, AUC was not relevant because it is very difficult to determine the long-term elimination of antibodies; hence, only AUD is given. In the P-HTIG plus placebo group, C_{max} was 0.313 ± 2.49 IU/ml and T_{max} was 4.46 ± 1.92 days. In the P-HTIG plus Td vaccine group, C_{max} was 15.2 ± 2.42 IU/ml and T_{max} was 18.80 ± 1.40 days. The times to reach the set concentration threshold (0.01 or 0.1 IU/ml) were similar for the two groups (P-HTIG plus Td vaccine or P-HTIG plus placebo). The times to reach the threshold of 0.01 IU/ml (minimum seroprotective level) were 1.12 ± 1.32 days (range, 1 to 2 days) for the P-HTIG plus placebo group and 1.00 day for the P-HTIG plus Td vaccine group. The times to reach the threshold of 0.1 IU/ml were 1.64 ± 1.62 days (range, 1 to 3 days) for the P-HTIG plus placebo group and 1.47 ± 2.13 days (range, 1 to 7 days) for the P-HTIG plus Td vaccine group. The distribution of subjects according to antibody titer classes (considering an absence of antitetanus antibodies at baseline) during the first 3 days after immunization was evaluated (Table 5). One hundred percent of subjects had minimum seroprotective levels (antibody titers, ≥0.01 IU/ml) after 2 days, while nearly 80% of subjects in both groups had seroprotective levels (antibody titers, ≥0.1 IU/ml) after 3 days.

To describe the antibody response after administration of P-HTIG alone or simultaneously with Td vaccine, a compartmental analysis with modelization was used. Fig. 3 presents the evolution, calculated according to the models described above, for the tetanus concentration evolution profiles for the two groups. During the first 6 days postinjection, the two curves were similar, and then the slope of the curve changed dramatically in the Td vaccine group, corresponding to the vaccine response. It was not possible to obtain values of this parameter for the Td vaccine group because of the brief period of evaluation. The values of C_{max} and T_{max} were close to the previous values; the values of C_{max} and T_{max} were 0.19 IU/ml and 3.57 days, respectively, for the P-HTIG plus placebo group (groups A and B) and 13.1 IU/ml and 19 days, respectively, for the P-HTIG plus Td vaccine group (groups C and D).

Antidiphtheria antibody titers were also determined, and the

TABLE 4. Mean pharmacokinetic parameters based on normalized data for P-HTIG plus placebo and P-HTIG plus Td vaccine groups

Group	AUD_{0-t} (IU · day/ml)[a,b]	C_{max} (IU/ml)[a]	T_{max} (days)[a]	Time (days) to the following antibody level[c]	
				0.01 IU/ml	0.1 IU/ml
P-HTIG plus placebo	6.11 ± 2.56 (2.11–27.8)	0.313 ± 2.49 (0.089–1.12)	4.46 ± 1.92 (3–14)	1.12 ± 1.32 (1.00–2.00)	1.64 ± 1.62 (1.00–3.00)
P-HTIG plus Td vaccine	390 ± 2.26 (79–1,263)	15.2 ± 2.42 (3.20–67.8)	18.8 ± 1.41 (14–42)	1.00 ± 0.00	1.47 ± 2.13 (1.00–7.00)

[a] Values are means ± SDs (minimum maximum values).
[b] AUD_{0-t}, AUD from time zero to time *t*.
[c] Values are means ± SDs (days).

TABLE 5. Distribution of normalized antibody titers from D1 to D3 postinjection in P-HTIG plus placebo group (groups A and B) and P-HTIG plus Td vaccine group (groups C and D)

Group and day after immunization	No. of subjects with the following antibody titer (IU/ml):			Total no. of subjects	% Subjects with titer of ≥0.01	% Subjects with titer of ≥0.1
	<0.01	0.01–0.1	≥0.1			
Groups A and B (n = 6)						
D1	1	3	2	6	83	33
D2	0	2	4	6	100	66
D3	0	1	5	6	100	83
Groups C and D (n = 13)						
D1	0	3	10	13	100	77
D2	0	5	8	13	100	62
D3	0	3	10	13	100	77

percentage of subjects with protective antibody titers (i.e., ≥0.1 IU/ml) increased from a baseline value of 42% to a value of 83% 42 days after vaccination.

DISCUSSION

To our knowledge, the clinical trial described here is the first to characterize the pharmacokinetic profile of human tetanus immunoglobulin (HTIG) and Td vaccine in combination in humans. In addition, we examined the safety of the new P-HTIG by thorough evaluation both of local reactions (during the 3 days following each injection) and of systemic reactions (during the entire course of the study, i.e., up to 42 days postinjection). There were no severe adverse events, particularly no case of immediate anaphylactic shock or late serum sickness reaction, reported after administration of the 500-IU dose of P-HTIG (the highest recommended dose). Fewer local reactions occurred at the P-HTIG injection site than at the Td vaccine injection site, and those were mild and short-lived. Rates of systemic reactions were similar in the four study groups. These reactions were mild and lasted no more than 2 days.

It is difficult to compare the adverse reaction rates of HTIG among different studies due to the variability in study designs.

Even though all subjects received their last tetanus booster dose more than 10 years prior to the start of the study, some high preimmunization antibody titers were found (range, 0.05 to 10.03 IU/ml). After treatment, serum tetanus antibody concentrations were above protective levels during the entire 42-day period of evaluation. There was a marked intersubject variability in the response to P-HTIG injection, which can be partially attributed to the variability of the titration method. However, we applied a procedure for determining the individual increase in antibody titers from the baseline due to the administration of P-HTIG either alone or in association with Td vaccine.

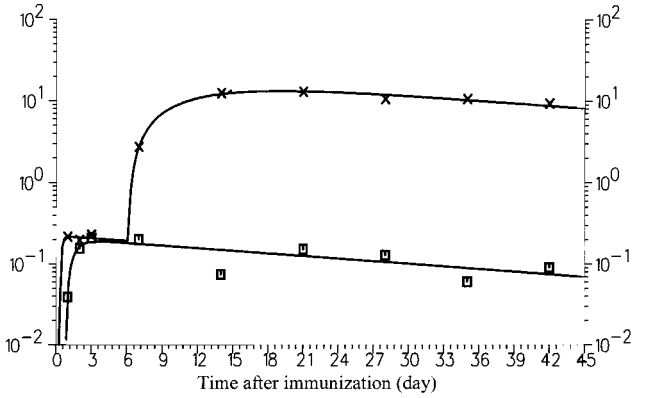

FIG. 3. Calculated tetanus concentration profile (log-linear scale; data normalized) following administration of P-HTIG alone (lower curve) or P-HTIG with Td vaccine (upper curve). Calculated as described in the text with the following values for the parameters: k_{el} = 0.0246, k_{01} = 15, k_a = 8.65, k_b = 0.175, t_{lag} = 0.865 days, t_{lag1} = 0.298 days, and t_{lag2} = 6.05 days.

The evolution of the tetanus antibody titers in the group that received P-HTIG alone and in the group that received P-HTIG plus Td vaccine was similar until D3 to D7. Likewise, modelization of antibody titers clearly showed similar profiles in the two groups until the seventh day. Our results demonstrate the absence of an early antitoxin response to the tetanus booster, similar to previous observations (23, 28, 33). We estimate that the formation of antibodies in response to vaccination occurs at about day 6. (An exact prediction cannot be made because of insufficient data between days 3 and 7.) Thus, treatment of tetanus-prone wounds with immunoglobulin is crucial, especially in light of the fact that the incubation period of tetanus can be as short as 3 to 4 days (27).

As a result of P-HTIG, 83% of subjects in the placebo group and 100% of subjects in the Td vaccine group reached the minimum seroprotective level (i.e., 0.01 IU/ml) by D1. Almost 80% of subjects in either group reached the 0.1 IU/ml tetanus antibody level after D3. The antibody titer most commonly considered to be seroprotective for tetanus is 0.01 IU/ml, but seroprotective levels probably vary between 0.01 and 0.1 IU/ml (17, 25).

Tetanus has been described in patients possessing antibody levels above 0.01 IU/ml (10, 22). In addition, the sensitivity of assay methods differs, because a tetanus antitoxin level of 0.01 IU/ml determined by in vivo assays such as the neutralization assay corresponds to a higher level of antibody determined by an in vitro assay, such as ELISA (9). A tetanus antibody titer of 0.1 IU/ml by ELISA is thus a safe estimate of seroprotection (7, 9).

Administration of the antigen and its antibodies simultaneously could lead to mutual neutralization. Comparative studies of simultaneous and deferred injection of tetanus vaccine and HTIG at a low dose of 250 IU (prophylaxis) (16, 28) or at a high dose of 45,000 IU (therapy) (36) do not show any statistically significant differences in the quality of the active immune response. However, Levine et al. (15) observed that concurrent administration of HTIG and vaccine induced a delay in the response to the vaccine. A study involving 119 healthy adults between 20 and 40 years of age with previous

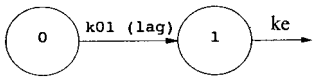

FIG. A1. Combined model for groups A and B.

histories of vaccination against tetanus (7) found reduced tetanus antibody titers after the simultaneous administration of HTIG and Td vaccine. However, the percentage of protective seroprevalence levels (>0.1 IU/ml) was unchanged.

In our study, there was a 10-fold rise in antitetanus antibody titers (unnormalized) from D0 to D28 (1.2 to 11.6 IU/ml, on average), which is similar to the rise found in a previous study with Td vaccine alone (11). On the basis of previous results (32) and on the average antibody titer of 10 IU/ml reported at D42 in our study, we anticipate that antibody levels will persist above 0.1 IU/ml for at least 20 years. Thus, if an interference between P-HTIG and Td vaccine exists, it has no clinically detectable effects. In addition, the postimmunization diphtheria antibody levels were satisfactory.

Although calculation of the elimination half-life of normalized antibody titers was not completely relevant in this study, we found a value of 28 days, which is in agreement with previously reported values (29, 34). The half-life of P-HTIG is much longer than that reported for antibodies of equine origin (7 to 14 days) (16, 34). All subjects in the P-HTIG group alone had protective antibody titers during the follow-up, which covered the therapeutic window, when a delay in the response to the vaccine may occur, such as among elderly (14) or unimmunized (20) people.

In conclusion, the new P-HTIG has a good safety profile. It induces seroprotective levels of tetanus antibodies beginning 1 to 2 days postadministration. No clinically significant interference with Td vaccine was reported. P-HTIG can be safely used for the effective management of tetanus-prone wounds.

APPENDIX

The log-linear plot of the concentration data shows a linear decline in the time interval after reaching a maximum value, suggesting that only a single distribution or elimination phase is detectable. Thus, a one-compartment open model was used to describe the distribution or elimination for both data sets. Differences between the curves are apparent until the maximum titers are reached. For groups A and B (P-HTIG plus placebo), a rapid increase in antibody concentration could be observed. Therefore, a simple, monoexponential input function was chosen. The combined model for groups A and B is shown in Fig. A1.

The parameters of the model for groups A and B (shown in Fig. A1) are $k_a = k_{01}$ (absorption rate constant), t_{lag} (associated lag time), and k_{el}, where 1 is the central compartment (site of measurement) and 0 is the absorption compartment. The model is described by a Bateman function, as follows:

$$C_t = y_0 \cdot \left(\frac{k_a}{(k_{el} - k_a)} \cdot e^{-k_a \cdot (t - t_{lag})} + \frac{k_a}{(k_a - k_{el})} \cdot e^{-k_{el} \cdot (t - t_{lag})} \right)$$

For groups C and D (P-HTIG plus Td vaccine), an initial increase in titers similar to that for groups A and B could be observed within the time interval of D1 to D3. On D7, a further increase in titers led to maximum concentrations approximately 20 days after treatment. Therefore, an input function with two separate pathways was chosen for this data set. The combined model is shown in Fig. A2.

The parameters of the model for groups C and D (shown in Fig. A2) are k_a (absorption rate constant for first pathway), t_{lag1} (associated lag time), k_b (absorption rate constant for second pathway), t_{lag2} (associated lag time), k_{01} (absorption rate common for both pathways), and k_{el}; 1 is the central compartment (site of measurement) and A, B, and 0 are the absorption compartments.

The model is described by a sum of two functions, one for each pathway, $C_t = Ca_t + Cb_t$, with

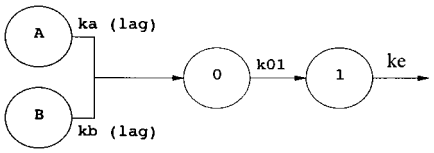

FIG. A2. Combined model for groups C and D.

$$Ca_t = y_0 \cdot a \cdot \left[\frac{k_a \cdot k_{01}}{(k_{01} - k_a) \cdot (k_{el} - k_a)} \cdot e^{-k_a \cdot (t - t_{lag1})} + \frac{k_a \cdot k_{01}}{(k_a - k_{01}) \cdot (k_{el} - k_{01})} \cdot e^{-k_{01} \cdot (t - t_{lag1})} + \frac{k_a \cdot k_{01}}{(k_a - k_{el}) \cdot (k_{01} - k_{el})} \cdot e^{-k_{el} \cdot (t - t_{lag1})} \right]$$

$$Cb_t = y_0 \cdot b \cdot \left[\frac{k_b \cdot k_{01}}{(k_{01} - k_b) \cdot (k_{el} - k_b)} \cdot e^{-k_b \cdot (t - t_{lag2})} + \frac{k_b \cdot k_{01}}{(k_b - k_{01}) \cdot (k_{el} - k_{01})} \cdot e^{-k_{01} \cdot (t - t_{lag2})} + \frac{k_b \cdot k_{01}}{(k_b - k_{el}) \cdot (k_{01} - k_{el})} \cdot e^{-k_{el} \cdot (t - t_{lag2})} \right]$$

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REFERENCES

1. **Anderson, S., A. Auquier, W. W. Hauck, D. Oakes, W. Vandaele, and H. I. Weisberg.** 1980. Statistical methods for comparative studies. John Wiley & Sons, Inc., New York, N.Y.
2. **Aronson, J. K., H. J. Dengler, L. Dettli, and F. Follath.** 1988. Standardization of symbols in clinical pharmacology. Eur. J. Clin. Pharmacol. **35:**1–7.
3. **Bytchenko, B.** 1966. Geographical distribution of tetanus in the world 1951–1960. Bull. W. H. O. **34:**71–104.
4. **Centers for Disease Control.** 1991. Update on adult immunization. Recommendations of the Immunization Practice Advisory Committee (ACIP). Morbid. Mortal. Weekly Rep. **40:**47–52, 67–71, 73, 77–81, 86–94.
5. **Cox, D. R.** 1972. Regression models and life-tables. J. R. Stat. Soc. B **34:** 187–20.
6. **Cox, N. H., and A. Forsyth.** 1988. Thiomersal allergy and vaccination reactions. Contact Dermatitis **18:**229–233.
7. **Dal-Ré, R., G. Angel, A. Gonzáles, and L. Lasheras.** 1995. Does tetanus immune globulin interfere with the immune response to simultaneous administration of tetanus-diphtheria vaccine? A comparative clinical trial in adults. J. Clin. Pharmacol. **35:**420–425.
8. **Falksveden, L., T. Bö, K. Hanngren, et al.** 1978. Tetanus immune globulin in clinical evaluation of a new plasma fractionation method. *In* Proceedings of the 5th International Conference of Tetanus.
9. **Galazka, A.** 1993. Expanded Programme on Immunization: the immunological basis for Immunization. Module 3: tetanus. WHO Documents WHO/EPI/GEN/93.12–93.19. World Health Organization, Geneva, Switzerland.
10. **Goulon, M., O. Girard, S. Grosbuis, J. P. Desormeau, and M. F. Capponi.** 1972. Les anticorps antitétaniques. Titrage avant séro-anatoxinothérapie chez 64 tétaniques. Presse Med. **45:**3049–3050.
11. **Harcus, A. W., A. E. Ward, J. St. C. Roberts, and K. A. Bryett.** 1989. An evaluation of diphtheria-tetanus (adult) vaccine in unselected human volunteers. J. Intern. Med. Res. **17:**262–267.
12. **Heinzel, G., R. Woloszczak, and P. Thomann.** 1993. TopFit 2.0. *In* Pharmacokinetic and pharmacodynamic data analysis system for the PC. G. Fischer Verlag, Stuttgart, Germany.
13. **Kaplan, E. L., and P. Meier.** 1958. Nonparametric estimation of incomplete observations. J. Am. Stat. Assoc. **53:**457–481.
14. **Kishimoto, S., S. Tomino, H. Mitsuya, H. Fujiwara, and H. Tsuda.** 1980. Age-related decline in the *in vitro* and *in vivo* synthesis of anti-tetanus toxid antibody in humans. J. Immunol. **125:**2347–2352.
15. **Levine, L., J. A. McComb, R. C. Dwyer, and W. C. Latham.** 1966. Active-passive tetanus immunization. N. Engl. J. Med. **27:**186–190.
16. **McComb, J. A., and R. C. Dwyer.** 1963. Passive-active immunization with tetanus immune globulin (human). N. Engl. J. Med. **268:**857–862.

17. **McComb, J. A.** 1964. The prophylactic dose of homologous tetanus antitoxin. N. Engl. J. Med. **270:**175–178.
18. **Melville-Smith, M. E., V. A. Seagroatt, and J. T. Watkins.** 1983. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. J. Biol. Stand. **11:**137–144.
19. **Miller, E., and M. Rush.** 1994. Immunity to diphtheria in adults in England. Br. Med. J. **308:**598.
20. **Myers, M. G., C. W. Beckman, R. A. Vosdingh, and W. A. Hankins.** 1982. Primary immunization with tetanus and diphtheria toxoids. JAMA **248:**2478–2480.
21. **Newton-John, H. F.** 1984. Tetanus in Victoria 1957–80. Med. J. Aust. **140:** 194–200.
22. **Passen, E. L., and B. R. Andersen.** 1986. Clinical tetanus despite a protective level of toxin neutralizing antibody. JAMA **255:**1171–1173.
23. **Porter, J. D. H., M. A. Perkin, M. J. Corbel, et al.** 1992. Lack of early antitoxin response to tetanus booster. Vaccine **10:**334–336.
24. **Prevots, R., R. W. Sutter, P. M. Strebel, S. L. Cochi, and S. Hadler.** 1992. Tetanus Surveillance—United States, 1989–1990. Morbid. Mortal. Weekly Rep. **41:**1–9.
25. **Regamey, R. H.** 1965. Gammaglobuline antitétanique humaine et séroprophylaxie du tétanos. Prog. Immunobiol. Stand. **2:**93–116.
26. **Rey, M., and E. Tikhomirov.** 1989. Non neonatal tetanus over the world, p. 506–518. *In* G. Nistico, B. Bizzini, B. Bytchenko, and R. Triau (ed.), Proceedings of the 8th International Conference on Tetanus. Pythagora Press, Rome, Italy.
27. **Rothstein, R. J., and F. J. Baker.** 1978. Tetanus. Prevention. JAMA **240:** 675–676.
28. **Rubbo, S. D.** 1966. Prophylaxis against tetanus. Principles on tetanus, p. 341–354. *In* L. Eckmann (ed.), Proceedings of the 2nd International Conference on Tetanus. Hans Huber Publishers Bern, Switzerland.
29. **Rubinstein, H. M.** 1962. Studies on human tetanus antitoxin. Am. J. Hyg. **76:**276–292.
30. **SAS.** 1990. SAS manuals, version 6. SAS Institute Inc., Cary, N.C.
31. **Sesardjic, D., and M. J. Corbel.** 1992. Testing for neutralising potential of serum antibodies to tetanus and diphtheria toxin. Lancet **340:**737–738.
32. **Simonsen, O.** 1989. Vaccination against tetanus and diphtheria. Dan. Med. Bull. **36:**24–46.
33. **Simonsen, L., O. Simonsen, K. Kjeldsen, and I. Heron.** 1989. The initial antitoxin response following revaccination, p. 394–397. *In* G. Nistico, B. Bizzini, B. Bytchenko, and R. Triau (ed.), Proceedings of the 8th International Conference on Tetanus. Pythagora Press, Rome, Italy.
34. **Smolens, J., A. B. Vogt, M. N. Crowford, and J. Stokes.** 1961. The persistence in the human circulation of horse and human tetanus antitoxins. J. Pediatr. **59:**899–902.
35. **Stiehm, E. R.** 1979. Standard and special human immune serum globulins as therapeutic agents. Pediatrics **63:**301–319.
36. **Ullberg-Olsson, K.** 1975. Active immunization against tetanus in man. III. Antibody response to vaccine injections in the presence of therapeutic doses of human tetanus immune globulin. A record of five cases. Eur. Surg. Res. **7:**305–314.
37. **Wells, M. A., A. E. Willer, J. S. Epstein, et al.** 1986. Inactivation and partition of human T-cell lymphotropic virus type III during ethanol fractionation of plasma. Transfusion **26:**210–213.
38. **World Health Organization.** 1996. Fighting disease and fostering development, p. 24. *In* WHO health report. World Health Organization, Geneva, Switzerland.